Blood-Brain Glucose Transfer In Diabetes Mellitus

Decreased Number of Glucose Transporters at Blood-Brain Barrier

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SUMMARY

This study describes the effects of streptozocin (STZ)-induced diabetes mellitus on the glucose-transporter system of the rat blood-brain barrier. Subcellular membrane fractions, i.e., plasma membranes and high- and low-density microsomes, were prepared from isolated brain microvessels derived from control and diabetic animals. The number of glucose transporters in each of the membrane fractions from both control and diabetic animals was determined by the D-glucose–inhibitable cytochalasin B–binding assay. The total number of glucose transporters was decreased by 43% in STZ-treated rats compared with controls (35 vs. 115 pmol/mg protein; $P < .05$). The glucose-transporter number in plasma membranes was decreased by 50%, in high-density microsomes by 38%, and in low-density microsomes by 45%. Incubation of isolated microvessels from control animals with 7 μM insulin for 30 min at 37°C led to a cycloheximide-sensitive 27% increase ($P < .05$) in the number of transporters in high-density microsomes. This insulin effect was significantly diminished to 15% in the diabetic animals ($P < .05$). In conclusion, *1*) STZ-induced diabetes decreases the glucose-transporter number in all subcellular membrane fractions derived from isolated rat brain microvessels, and *2*) the insulin-induced increase in de novo synthesis of glucose transporters in brain microvessels is diminished in these chronically diabetic animals.

DIABETES 1986; 35:1181–84.

Brain microvessel endothelial cells form a blood-brain barrier that restricts the exchange of solutes between blood and brain.[1] Glucose is transported across the cerebral capillary endothelium by carrier-mediated facilitated diffusion.[2–4] Neuroglycopenia occurs in diabetics when plasma glucose values fall to hypoglycemic levels, and it has been suggested that relative cerebral hypoglycemia can occur in the absence of chemical hypoglycemia, when glucose levels drop from hyperglycemic to euglycemic values; this suggests insufficient capacity for glucose transport across the blood-brain barrier.[5,6] Indeed, with different methodologies, i.e., extraction of labeled glucose from brain, glucose conversion to CO_2, and the Oldendorf brain uptake index method, it has been shown that the maximum in vivo transport capacity for glucose is decreased in chronically hyperglycemic animals.[7–9] To gain insight into the cellular events accompanying this phenomenon, we investigated the effects of streptozocin (STZ)-induced insulin-deficient diabetes mellitus on the number and subcellular distribution of glucose transporters at the blood-brain barrier.

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Received for publication 27 May 1986 and in revised form 7 July 1986.

MATERIALS AND METHODS

One hundred thirty-three male Sprague-Dawley rats were divided into two groups. The experimental group was rendered hyperglycemic by a single injection of STZ (50 mg/kg body wt i.p.). Both groups ate rat chow ad libitum and were housed in the same way for 4 wk. Plasma glucose levels and other parameters were measured and are shown in Table 1. The animals were decapitated, and microvessels from cortex were prepared by a modification[10] of the method of Goldstein et al.[11] according to the following procedure. Subcellular membrane fractions from isolated microvessels were also prepared according to the following procedure. After isolation from cerebral cortex microvessels were centrifuged at 500 × g_{max} for 5 min.[5,6] The resulting pellet was resuspended in ice-cold 20 mM Tris, 1 mM EDTA, and 250 mM sucrose (TES) buffer, pH 7.4, containing 2.5 μg/ml aprotinin, leupeptin, and pepstatin. The microvessels were homogenized by sonication (2 × 2 min) on ice with a Branson model 200 cell disruptor (Heat Systems-Ultrasonics, Farmingdale, KY). The resulting homogenate was centrifuged at 17,000 × g_{max} for 30 min. The supernatant was saved for the preparation of microsomal membrane fractions. The pellet, which contained plasma membranes and mitochondria, was resuspended

TABLE 1
Metabolic features of streptozocin-diabetic and control rats

	Control group (N = 69)	Streptozocin group (N = 64)
Body weight before treatment (g)	233.4 ± 2.1	228.3 ± 1.8
Body weight at death (g)	368.0 ± 8.9	273.2 ± 5.3
Urine glucose at death (%)	NM	>5
Plasma glucose at death (mmol/L)	8.8 ± 0.2	34.5 ± 1.1

The animals received a single injection of streptozocin (50 mg/kg body wt i.p.) 4 wk before experiments were performed. Values are expressed as means ±SE. NM, not measurable.

into TES buffer, layered on a Tris (20 mM) plus EDTA (1 mM) plus 41% sucrose cushion, and centrifuged at 101,000 × g_{max} for 1 h. Plasma membranes, which band at the 41% sucrose-TES interface, were collected by aspiration with a Pasteur pipette and diluted with TES buffer. The purified plasma membranes were pelleted by centrifugation at 48,000 × g_{max} for 45 min. The resulting pellet was washed in TES buffer and collected by centrifugation at 17,000 × g_{max} for 30 min. The plasma membranes were resuspended in TES and stored at −80°C. The initial supernatant was centrifuged at 48,000 × g_{max} for 30 min, yielding a pellet of high-density microsomes. The supernatant was then centrifuged at 430,000 × g_{max} for 90 min, yielding a pellet of low-density microsomes. All pellets were resuspended in TES buffer, pH 7.4, and stored at −80°C.

Protein was determined by the method of Lowry et al.[14] as modified by Peterson.[15] Sodium fluoride–stimulated adenylate cyclase activity was measured by the method of Salomon et al.[16] Rotenone-insensitive NADH-cytochrome c reductase activity was determined by the method of Dallner et al.[17] *N*-acetyllactosamine synthase activity was determined by the method of Fleisher.[18] Phase-contrast microscopy revealed that the isolated microvessels were virtually free of contamination by glial or neuronal tissue. Alkaline phosphatase, which is primarily located within capillaries, was measured in microvessel preparations,[12] and enzyme activity was enriched 25 ± 1.2-fold (mean ± SE, N = 3) when compared with the cerebral cortex homogenate; these values are comparable with those reported in the literature.[11,13] As described elsewhere (S. Matthaei, J. M. Olefsky, T. W. Garvey, and R. Horuk, unpublished observations), compared with homogenate, the membrane-fractionation method leads to ~33-fold enrichment in adenylate cyclase in plasma membranes, ~21-fold increase in cytochrome c reductase in high-density microsomes, and ~6.5-fold increase in galactosyltransferase activity in low-density microsomes.

The number of D-glucose–inhibitable cytochalasin B–binding sites in the subcellular fractions was determined as previously described.[19]

RESULTS

By measuring D-glucose (500 mM)–inhibitable [^{3}H]cytochalasin B binding to the membrane fractions,[20] we estimated the number of glucose transporters in brain microvessel subcellular fractions from both control and STZ-induced diabetic rats with Scatchard plots of the [^{3}H]cytochalasin B–binding data. The results shown in Fig. 1 indicate that STZ-induced diabetes mellitus causes a decrease in glucose-transporter number in brain microvessel subcellular fractions by 50% in plasma membranes, 38% in high-density microsomes, and

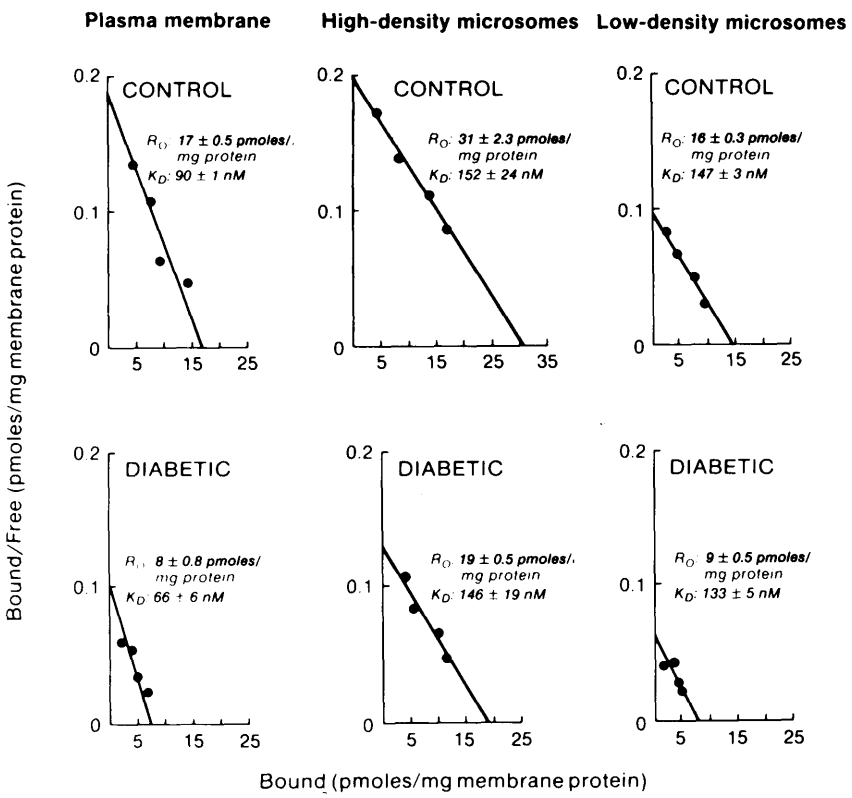

FIG. 1. Scatchard analyses of equilibrium [^{3}H]cytochalasin B binding to isolated brain microvessel subcellular fractions derived from diabetic and control rats. Cytochalasin B binding was measured at 4 cytochalasin B concentrations in absence or presence of 500 mM D-glucose.[20] Derived Scatchard plots were constructed by subtracting (along radial axes of constant free cytochalasin B concentrations) each curve obtained with D-glucose from its respective curve obtained without D-glucose. R_o, D-glucose–inhibitable cytochalasin B–binding sites in picomoles per milligram membrane protein; K_D, dissociation constant in nM. Means (±SE) of 4 separate cytochalasin B–binding assays, each done with 1 control and 1 diabetic membrane preparation studied in parallel.

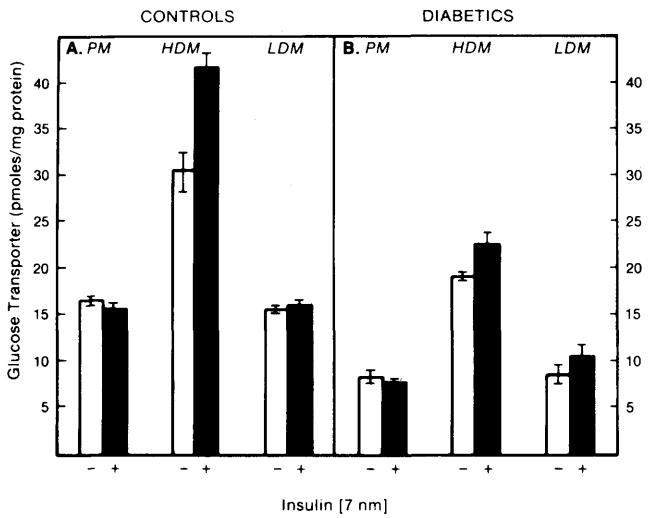

FIG. 2. Effect of insulin on the distribution of glucose transporters in rat brain microvessel subcellular membrane fractions from diabetic and control rats. Isolated microvessels were incubated with and without 7 nM insulin for 30 min in 30 ml of Krebs-Ringer-Hepes (25 mM) bicarbonate (1%) buffer containing 4% BSA at 37°C. Subcellular membrane fractions [plasma membranes (PM), high-density microsomes (HDM), and low-density microsomes (LDM)] were prepared as described in MATERIALS AND METHODS. Binding sites have been assessed by D-glucose–inhibitable cytochalasin B–binding assay as described in the legend of Fig. 1. Data represent means (±SE) of 4 separate cytochalasin B–binding assays, each done with control and diabetic membranes studied in parallel.

45% in low-density microsomes ($P < .05$). The number of glucose transporters in all membrane fractions combined was decreased by 43% (115 pmol/mg protein in controls vs. 35 pmol/mg protein in STZ-treated animals; $P < .05$).

As seen in Fig. 2, incubation of isolated microvessels from control animals with 7 nM insulin for 30 min at 37°C did not significantly alter the number of glucose transporters in plasma membranes and low-density microsomes but did increase the number of transporters in high-density microsomes by 27% ($P < .05$). Pretreatment of microvessels with cycloheximide (10 μg/ml) for 60 min before insulin completely inhibited the increase in high-density microsome [^{3}H]cytochalasin B–binding sites (Table 2), suggesting that this effect of insulin involved de novo synthesis of glucose transporters. Figure 2 also shows that the stimulatory effect of insulin to increase high-density microsome glucose transporters was blunted in the STZ-induced diabetic animals. Insulin induces a slight increase in the number of glucose transporters in high-density microsomes from the diabetic animals, but the increase is significantly lower than in control high-density microsomes (27% increase in controls vs. 15% in diabetic rats; $P < .05$). Insulin treatment did not significantly alter the dissociation constant (K_D) for [^{3}H]cytochalasin B binding to the different membrane fractions (data not shown).

DISCUSSION

Karnieli et al.[22] investigated the effects of STZ-induced diabetes mellitus on the glucose-transporter system in isolated rat adipocytes. In adipocytes, insulin stimulates glucose transport by inducing a rapid and reversible translocation of glucose transporters from an intracellular membrane pool associated with the low-density microsomal fraction to the plasma membrane.[20,21] Karnieli et al.[22] found a depletion of glucose transporters in the low-density microsomes consistent with the decrease in fat cell glucose-transport activity and the insulin resistance in these animals. Betz et al.[23] have shown that insulin did not acutely stimulate 3-*O*-methylglucose uptake by isolated brain microvessels. We have confirmed these results using 2-deoxyglucose as substrate (data not shown). Thus, insulin does not appear to have an acute effect on glucose uptake at the blood-brain barrier.

The findings presented here indicate that in the basal state, there are already many glucose carriers on the cell surface, and insulin does not acutely alter the subcellular distribution of transporters. However, insulin appears to stimulate de novo synthesis of glucose transporters in brain microvessels, as shown by the insulin-mediated increase in high-density microsome glucose transporters. This effect is diminished in the diabetic state and may underlie the decreased number of transporters in microvessel membrane fractions from these animals. The depletion of blood-brain glucose transporters is consistent with earlier in vivo studies that showed decreased brain glucose uptake and metabolism in experimental diabetic animals.[7–9] Whether it is the insulin deficiency, the hyperglycemia, both together, or some other aspect of the chronic diabetic state that leads to decreased brain microvessel glucose transporters cannot be determined from current studies. However, we favor the role of insulin deficiency, because non-insulin-mediated glucose uptake (predominantly central nervous system) is normal in hyperglycemic non-insulin-dependent diabetic subjects,[24] as are estimates of brain glucose extraction in insulin-treated but still hyperglycemic subjects.[25] We conclude that STZ causes a diabetic state associated with fewer glucose-transport proteins in brain microvessels, which may result in reduced capacity of glucose transfer across the blood-brain barrier. This phenomenon may contribute to the development of neuroglycopenia in diabetes.

ACKNOWLEDGMENTS

We thank Linda McLaren for technical assistance during this work and Elizabeth Martinez and Cleon Tate for expert secretarial assistance in the preparation of this manuscript.

This study was supported by NIH Grants AM-33649 and

TABLE 2
Effect of cycloheximide on microvessel glucose transporters in nondiabetic rats

	Plasma membranes	High-density microsomes	Low-density microsomes
Basal	18 ± 3	27 ± 3	21 ± 5
Insulin	19 ± 3	44 ± 2	25 ± 2
Cycloheximide	19 ± 2	28 ± 2	23 ± 2

Basal cells were incubated for 90 min with no additions; insulin cells were incubated for 60 min with no additions and then for 30 min with 7 nM insulin; cycloheximide cells were incubated with 10 μg/ml cycloheximide for 60 min and then with 7 nM insulin for 30 min. Glucose-transporter numbers were assessed by D-glucose–inhibitable cytochalasin B–binding assay. Numbers are given as pmol/mg membrane protein. Results are means (±SE) of 3 separate preparations assayed once each.

AM-33651 from the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases and by the Medical Research Service of the Veterans Administration Medical Center, San Diego, California.

S.M. is a recipient of a grant from the Deutsche Forschungsgemeinschaft (MA 985/1-1).

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